Amendment and Response

Serial No.: 09 701,947

Confirmation No.: 9854 Filed: December 5, 2000.

For: STABILIZED BIOACTIVE PEPTIDES AND METHODS OF IDENTIFICATION, SYNTHESIS AND

USE

Amendments to the Specification

Please replace the paragraph beginning at page 9, line 15 as follows:

Figure 1 shows the control region (SEQ ID NO:1) of the wild-type lac operon from the auxiliary operator O3 through the translational start of the lacZ gene. DNA binding sites include the operators O3 and O1 (both underlined), catabolite gene activator protein (CAP) (boxed), the -35 site (boxed), and the -10 site (boxed), while important RNA and protein sites include the LacI translation stop site (TGA), the +1 lacZ transcription start site, the Shine Dalgarno (SD) ribosome binding site for lacZ, and the LacZ translation start site (ATG).

Please replace the paragraph beginning at page 9, line 22 as follows:

Figure 2 is a map of plasmid pLAC11. The unique restriction sites and the base pair at which they cut are indicated. Other sites of interest are also shown, including Tet (98-1288), Rop (1931-2122), origin of replication (ori) (2551-3138), Amp (3309-4169), and *lac*PO (4424-4536).

Please replace the paragraph beginning on page 35, line 1 as follows:

Construction of the pLAC11, pLAC22, and pLAC33 expression vectors. To construct pLAC11, primers #1 and #2 (see Table 5) were used to polymerase chain reaction (PCR) amplify a 952 base pair (bp) fragment from the plasmid pBH20 which contains the wild-type lac operon. Primer #2 introduced two different base pair mutations into the seven base spacer region between the Shine Dalgarno site and the ATG start site of the lacZ which converted in from AACAGCT to AAGATCT thus placing a Bgl II site in between the Shine Dalgarno and the start codon of the *lacZ* gene. The resulting fragment was gel isolated, digested with Pst I and EcoR I, and then ligated into the 3614 bp fragment from the plasmid pBR322-Aval which had been digested with the same two restriction enzymes. To construct pBR322-Aval, pBR322 was digested

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with Aval, filled-in using Klenow, and then religated. To construct pLAC22, a 1291 bp Neo I, EcoR I fragment was gel isolated from pLAC21 and ligated to a 4361 bp Neo I, EcoR I fragment which was gel isolated from pBR322/Nco I. To construct pLAC21, primers #2 and #3 (see Table 5) were used to PCR amplify a 1310 bp fragment from the plasmid pMS421 which contains the wild-type *lac* operon as well as the *lacI*^q repressor. The resulting fragment was gel isolated, digested with EcoR I, and then ligated into pBR322 which had also been digested with EcoR I. To construct pBR322/Nco I, primers #4 and #5 (see Table 5) were used to PCR amplify a 788 bp fragment from the plasmid pBR322. The resulting fragment was gel isolated, digested with Pst I and EcoR I, and then ligated into the 3606 bp fragment from the plasmid pBR322 which had been digested with the same two restriction enzymes. The pBR322/Nco I vector also contains added Kpn I and Sma I sites in addition to the new Neo I site. To construct pLAC33, a 2778 bp fragment was gel isolated from pLAC12 which had been digested with BsaB I and Bsa I and ligated to a 960 bp fragment from pUC8 which had been digested with Afl III, filled-in with Klenow, and then digested with Bsa I. To construct pLAC12, a 1310 bp Pst I, BamH I fragment was gel isolated from pLAC11 and ligated to a 3232 bp Pst I, BamH I fragment which was gel isolated from pBR322.

Please replace the paragraph beginning at page 42, line 20 through page 43, line 16 as follows:

The data presented in Table 7 are the number of Kan^R transductants that were obtained from the different MC1061 derivative strains when they were transduced with a P1 lysate prepared from strain ALS598 which harbored a Tn10dKan transposon insertion. Overnights were prepared from each of these strains using either rich medium to which glucose was added at a final concentration of 0.2% (repressed conditions) or rich medium to which IPTG was added at a final concentration of 1 mM (induced conditions). The overnights were then diluted 1 to 10 into the same medium which

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contained CaCl2 added to a final concentration of 10 mM and aerated for two hours to make them competent for transduction with P1 phage. Cells were then spectrophotometrically normalized and <u>0.1 ml aliquots of cells at an OD₅₅₀ of 5 aliquots of 5 OD550 cell equivalents in a volume of approximately 0.1 ml were transduced with 0.1 ml of concentrated P1 lysate as well as 0.1 ml of P1 lysates that had been diluted to 10⁻¹, 10⁻², or 10⁻³. 0.2 ml of 0.1M Sodium Citrate was added to the cell/phage mixtures and 0.2 ml of the final mixtures were plated onto Rich Kanamycin plates and incubated overnight at 37°C. The total number of Kan^R colonies were then counted. ALS225 recA⁺ data points were taken from the transductions which used the 10⁻³ diluted phage, while ALS514 recA⁻ data points were taken from the transductions which used the concentrated phage. The data points for ALS515 recA⁻ pCyt-3-recA grown under repressed conditions were taken from the transductions which used the concentrated phage, while the data points for ALS515 recA⁻ pCyt-3-recA grown under induced conditions were taken from the transductions which used the 10⁻³ diluted phage.</u>